1. Title of the Research proposal / Synopsis

Unveiling the Interplay: Exploring Nanoparticles Impact on Drug-Protein Interactions.

2. Introduction:

Nanomaterial, whether occurring naturally, incidentally, or manufactured, is defined by the presence of unbound or aggregated particles, with external dimensions ranging from 1 to 100 nm for ≥50% of the particles, as per the number size distribution [1]. Recognizing the crucial role of nanomaterial-mediated technologies is paramount in conceptualizing the future modern lifestyle. Nano's Midas touch is discernible, leaving no facet of human life untouched. Numerous studies highlight the repercussions of the increased use of nanoparticles (NPs) on the body's normal Absorption, Distribution, Metabolism, and Excretion (ADME) system, affecting the drug-carrying affinity of common serum albumin protein and the activity of other biomolecules.

In the past decade, experimental evidence has shown that particles injected into biological fluids rapidly (<0.5 min) form a "corona" on their surface, composed of biomolecules like enzymes, proteins, peptides, and amino acids [2]. Dawson et al. pioneered the study of protein corona in 2007, analyzing the proteins associated with nanoparticles (NPs) across various bodiy fluids with different binding affinities[3]. The concept evolved into the "biomolecular corona," the dynamic formation process depends on NPs' unique properties, including size, surface chemistry, ligands, nature, shape, charge, surface curvature, and environmental factors [4]. The nature of the NPs biomolecular corona significantly influences their biological fate, affecting cellular uptake, immunological response, and biodistribution. Understanding NP protein corona formation is crucial due to the increasing number of applications in nanobiomedicine. In the food industry, titanium dioxide (TiO_2) nanoparticles, used for brightness, form protein coronas with amphiphilic food items. Thermal modifications impacted protein coronas on TiO_2 nanoparticles, with heat-sensitive proteins like glutenin showing decreased binding

affinity at high temperatures, while heat-resistant proteins like gliadin were less affected [5].

3. Scope of the work

This fundamental research on the protein corona is vital for the expanding use of NPs in nanotechnology and nanomedicine, shedding light on intricate dynamics and interactions.

Navigating the outcomes of past research on protein corona raises several critical challenges:

1. **Identifying Key Proteins in Physiological Behavior:** Determining the specific proteins within the protein corona that play a pivotal role in the physiological behavior of nanoparticles (NPs). Which components of the protein corona predominantly influence the observed physiological responses of NPs?
2. **Predicting Future Interactions with Organisms:** Assessing the potential impacts and predicting future interactions of the components within the protein corona with organisms. What are the ramifications of these interactions on biological systems?
3. **Significance of the Soft Corona in Real-Life Conditions:** Evaluating the importance of the soft corona in actual biological conditions. Does the loosely bound protein corona hold significance in real-life biological contexts?
4. **Adequacy of Techniques for In Vivo and Ex Vivo Observations:** Scrutinizing the sufficiency of existing techniques for observing protein corona formation, both in vivo and ex vivo. Are the current methodologies robust enough to capture the dynamic process of protein corona formation in diverse biological environments?

Addressing these challenges is crucial for advancing our understanding of the intricate interplay between nanoparticles and biological systems, paving the way for more informed and effective applications in nanobiomedicine.

In the realm of nanoparticle-mediated bioconjugates, our knowledge is disproportionately centered around a few proteins like HAS and BSA, leaving significant gaps. The complexity deepens when considering the body's plasma, housing approximately 3500 proteins with diverse interactions with nanoparticles [6]. This knowledge gap raises concerns about the potential health implications for future generations. To fill this void, our proposed study strategically delves into lesser-explored proteins and enzymes, such as Lysozyme and Fibrinogen. Utilizing in-silico methods and various spectroscopic techniques, this work aims to systematically decipher the intricate interactions of plasma proteins with drugs. The ultimate objective is to unravel the complexities, bridge knowledge gaps, and illuminate potential health implications arising from these interactions.

4. Plan of the work & Methodology to follow

This Ph.D. journey seeks to delve into the intricacies the interplay between drugs and plasma proteins using in-silico and spectroscopic studies.

1. **Spectroscopic Exploration:** Utilizing spectroscopic analysis to gain profound insights into the interaction nuances between drugs and plasma proteins.
2. **Comprehensive Data Validation:** Rigorously validating in-silico predictions through experimental corroboration, aiming to unravel key outcomes:
    - Understanding the Mechanism of Binding of drugs with plasma proteins.
    - Identifying structural changes in native protein confirmation post-interaction with diverse drugs.
    - Precise calculation of thermodynamic parameters ΔG, ΔH, ΔS and defining the nature of interactions.

- Interpreting decay profiles of fluorophore quenching data to discern static or dynamic binding based on spectral profile changes.
- Precisely identifying key amino acid interactions in drug-protein complexes.

3. **Dynamic Study of Protein Corona Formation:** Investigating the nuanced formation of a protein corona, elucidating the type and stability of this complex.
4. **Effect of Nanoparticles:** Assessing the impact of nanoparticles on the interaction between drugs and plasma proteins.

   Strategically synthesizing diverse experiments, we're unlocking crucial insights for advanced nanomedicine. Our focus: precision drug delivery and enhanced therapeutic efficacy, shaping the future of nanomedicine evolution.

Methodology: In-Silico Investigation of Protein-Drug Interactions

Objective: Conduct a comprehensive exploration of interactions between protein residues and drugs through advanced in-silico methodologies.

1. Protein Retrieval and Active Site Prediction:

- **Tools:** RCSB - PDB, Discovery Studio, CASTp
- **Procedure:** Retrieve relevant protein structures and predict active sites for subsequent analysis.

2. Ligand Selection and Dataset Preparation:

- **Sources:** PubChem, ChemSpider, Open Babel
- **Procedure:** Select antibacterial drugs, prepare ligand datasets for in-silico exploration.

3. Drug Likeness and Ligand Screening (Lipinski Rule of 5):

- **Tools:** DriLiTo, ADMET-SAR, Swiss-ADME
- **Procedure:** Assess drug likeness, screen ligands based on Lipinski Rule of 5 for further consideration.

4. Ligand Property Prediction and Screening:

- **Tools:** DriLiTo, ADMET-SAR, Swiss-ADME
- **Procedure:** Predict and screen ligand properties to ensure compatibility with the study objectives.

5. Protein and Ligand Preparation (ADTDocking in Autodock Vina):

- **Tool:** Autodock Vina
- **Procedure:** Prepare proteins and ligands for docking simulations using Autodock Vina.

6. Docking :

- **Platform:** Autodock, Autodock Vina
- **Procedure:** Conduct docking simulations to explore molecular interactions between proteins and ligands.

7. Protein-Ligand Residue Interaction Analysis (LigPlot):

- **Tool:** LigPlot
- **Procedure:** Analyze and visualize protein-ligand residue interactions to gain insights into binding patterns.

8. Poses Analysis:

- **Platform:** PyMol
- **Procedure:** Analyze docking poses for a deeper understanding of the spatial orientation of proteins and ligands.

9. Molecular Dynamics Simulation:

- **Software:** GROMACS
- **Procedure:** Perform molecular dynamics simulations to simulate the dynamic behavior of protein-ligand complexes.

10. Analysis of Simulation Plots:

- **Objective:** Scrutinize and interpret molecular dynamics simulation plots to unravel dynamic interactions over time.

Reporting and Documentation:

- **Objective:** Compile findings, insights, and analyses into a comprehensive report. Document the entire in-silico investigation journey, from initial data retrieval to dynamic simulation analyses. Ensure clear communication of methodologies and results for robust scientific interpretation.

Integrating diverse experiment findings for conclusive insights, our approach aims to contribute pivotal knowledge for advanced nanomedicine development. Focused on optimizing drug delivery and enhancing therapeutic efficacy, this synthesis deepens our understanding of drugs, plasma proteins, and nanoparticles. The goal is to propel nanomedicine evolution for precision and efficacy in drug delivery and treatment.

5. REFERENCES

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